REMARKS

I. Status of the Claims

Claims 62-83 are pending in the instant application. Claims 63-65, 67-74, 77-78, and 80 have been canceled without prejudice to the Applicant's right to pursue claims of the same or similar subject matter in a duly filed continuing application. Claims 62, 66, 75-76, 79, and 81 have been amended to more particularly recite the subject matter Applicant regards as the invention. The amendments to the claims do not add new matter.

II. The rejection of claims 66-83 under 35 USC §112, first paragraph, may be withdrawn.

Claims 66-83 were rejected under 35 USC §112, first paragraph, for assertedly failing to comply with the written description requirement. More specifically, the Examiner asserted that the specification does not support the combination of elements currently claimed, i.e., Lys to Arg substitutions at positions 17, 35, and 41, in combination with another substitution, i.e., introduction of a Lys in an internal core helix. The Examiner further asserts that the specification does not provide written description for an amino acid change in an internal core helix because the specification teaches that such internal helices are essential for structural integrity. The Examiner further asserts that there is no suggestion of modifying an internal core helix residue with PEG. Applicant respectfully traverses.

First, Applicant points out that claims 66 and 75 (and thus claims dependent therefrom) recite G-CSF analogs having lysine residues at positions 17 and 41 modified and there is no dispute that these modifications are not described. Importantly, Table 5 shows that modification of these residues results in an analog with biological activity.

Moreover, contrary to the Examiner's assertion that the specification teaches that mutations should not be made in the helical regions (Office action, pages 3-4 and citing page 66 of the specification), page 66, lines 9 through 26, of the specification teaches that certain specific internal hydrophobic residues are essential for structural integrity:

> Generally, for the G-CSF internal core--the internal four helix bundle lacking the external loops-- the hydrophobic internal residues are essential for structural integrity. For example, in helix A, the internal hydrophobic residues are (with methionine

> being position 1) Phe_{14}, Cys_{18}, Val_{22}, Ile_{25}, Ile_{32} and Leu_{36}. Generally, for the G-CSF internal core--the internal four helix bundle lacking the external loops-- the hydrophobic internal residues are essential for structural integrity. For example, in helix A, the internal hydrophobic residues are (with methionine being position 1 as in FIG. 1, Seq.ID. No.2) Phe_{14}, Cys_{18}, Val_{22}, Ile_{25}, Ile_{32} and Leu_{36}. The other hydrophobic residues (again with the met at position 1) are: helix B , Ala_{72}, Leu_{76}, Leu_{79}, Leu_{83}, Tyr_{86}, Leu_{90} Leu_{93}; helix C, Leu_{104}, Leu_{107}, Val_{111}, Ala_{114}, Ile_{118}, Met_{122}; and helix D, Val_{154}, Val_{158}, Phe_{161}, Val_{164}, Val_{168}, Leu_{172}.

These specific residues interact with other hydrophobic residues in the internal region formed by association of the four helices. Thus, these residues in individual helices are required to maintain the core structural integrity, while the other (i.e., "non-core" forming) residues in each helix do not interact in the hydrophobic core. The worker of ordinary skill in the art will appreciate that the "non-core" forming residues can thus be modified to the extent that the substitution does not rotate an essential residue out of the hydrophobic core. This position is further supported in the specification at page 16, lines 17 through 22 which states:

> Without knowledge of the overall three dimensional structure, preferably to the atomic level as provided herein, one could not forecast which alterations within this hydrophobic internal region would result in a change in the overall structural conformation of the molecule.

Thus, analogs with modifications in the helical core are clearly contemplated, but caution is advised against modifying certain specific helix residues. This fact is evidenced by disclosure in the specification that lysine at position 17, which is located in helix A but not in the hydrophobic core, can be substituted and the resulting analog still possesses biological activity. (See, e.g., variant 34 in Table 5 which retains G-CSF activity)

The Applicant notes that the Examiner does not reject the claimed subject matter as it relates to recitation of a modification wherein at least one amino acid sequence in an external loop is altered to include one or more lysine residues wherein one or more of the lysines is covalently modified with polyethylene glycol. For the sake of providing a complete response, Applicant points to the specification at page 18, lines 7 through 24, where modifications of this type are described.

The discussion above demonstrates that each limitation recited in the rejected claims is described in the specification. That each is described individually is not meant that

the only analogs "described" in the specification must have only one of the recited modifications. To wit, at page 7, line 33 through page 8, line 4, the specification discloses:

> With the presently provided knowledge of areas of hydrophobicity/-philicity, one may forecast with substantial certainty which **changes** to the G-CSF molecule will affect the overall structure of the molecule.

Similarly, at page 8, lines 23 through 27, the specification teaches:

> These analogs are molecules which have more, fewer, different or modified amino acid residues from the G-CSF amino acid sequence. The modifications may be by addition, substitution or deletion of **one or more amino acid residues**.

Certainly then, it will be appreciated that the invention contemplates analogs having more than one of the different types of modifications disclosed.

Thus, the specification discloses a large number of modifications that can be introduced into the wild-type G-CSF amino acid sequence to produce G-CSF analogs according to the invention. The specification also discloses analogs comprising one or more amino acid residue modifications. The specification further discloses each of the modifications recited in the rejected claims. Accordingly, the specification demonstrates that Applicant was in fact in possession of the subject matter of the rejected claims, as well as any G-CSF analog having any single or combination of modifications described. The Applicant therefore submits that the rejection under §112, first paragraph, may properly be withdrawn.

III. The rejection of claims 62-83 under 35 USC §112, first paragraph, may be withdrawn.

Claims 62-83 were rejected under 35 USC §112, first paragraph, for assertedly failing to provide enablement for G-CSF variants having any amount of hematopoietic activity and having any number of alterations in the internal or external loops. More specifically, the Examiner asserted that "given the scope of the claims, the single working example and the provided guidance are not found sufficient to enable the making and use of G-CSF variants to the full extent as claimed." (Office action, page 5) Applicant respectfully traverses.

I. Description of the invention

The present invention relates to specific G-CSF analogs which are described in detail in the specification. Each analog is prepared from naturally-occurring G-CSF (which also includes an amino terminal methionine residue), the structure of which is set out in the specification in the most precise three-dimensional structure determined as of the priority date of the instant application. Indeed, this family of applications made available for the first time the three-dimensional coordinates for every atom in wild-type G-CSF, thereby allowing for the visualization of the spatial relationship of every atom in G-CSF to every other atom in the molecule. (See, e.g., Figure 5) With this information, the worker of ordinary skill would reasonably be able to predict how a change in any one or any combination of amino acids will affect the overall protein structure. See, for example, discussion in the specification at page 13, line 9, through page 14, line 28, which teaches that not only can the native G-CSF molecule be visualized in three dimensions, but that the analogs described and claimed can also be visualized without having to produce the recombinant molecule.

In addition to disclosing the atomic coordinates for each G-CSF atom, the specification teaches that G-CSF biological activity is dependent upon binding to its cognate receptor. (See page 15, line 29, to page 16, line 1) Exhaustive description is also provided regarding specific amino acid residues in the molecule that participate and/or are required for receptor binding, either through directly interacting with the receptor or by indirectly interacting with other residues to maintain structural integrity and allow for receptor binding. (See, e.g., page 63, line 8, to page 72, line 24) Combining this teaching of residues necessary for biological function with the precise disclosure of structure further reduces the unpredictability associated with amino acid modification to a protein that would arise with knowledge of nothing more than the protein's primary amino acid sequence.

Even further, the specification applies the combination of these teachings in preparation of a myriad of G-CSG analogs, describing many in terms of structural and functional changes. For example, Table 5, page 62, shows how HPLC elution profiles differ after sequence modification, where a difference in the elution time correlates with overall structure change. Table 5 also shows biological activity of various analogs relative to wild-type G-CSF.

The combination of these teaching is summaraized at page 72, lines 17 through 24:

> Knowledge of the three dimensional structure and correlation of the composition of G-CSF makes possible a systematic, rational method for preparing G-CSF analogs. The above working examples have demonstrated that the limitations of the size and polarity of the side chains within the core of the structure dictate how much change the molecule can tolerate before the overall structure is changed.

II. Patentability argument

The Examiner states that "given the scope of the claims, the single working example and the provided guidance are not found to be sufficient to enable the making and use of G-CSF variants to the full extent as claims." (Office action, page 5) Moreover, the Examiner relies on the disclosure of Bowie, et al., to support the contention that "it is noted that the literature indicates that the art of protein modification is highly unpredictable." (Id.)

Addressing the last point first, Applicant submits that the disclosure of the atomic coordinates of every atom in G-CSF significantly reduces the unpredictability of protein modification. In other words, the skilled worker having nothing but the knowledge of the primary amino acid sequence of a protein would not understand the spatial and/or interactive relationship between individual amino acid residues in the context of maintaining overall structure and biological activity. The instant disclosure is without a doubt more enlightening. In addition to the tremendous advantage offered by providing the atomic coordinates of every atom, the specification teaches which regions and residues in the molecule (and they are not simply continuous and co-linear) participate in receptor binding, either directly or indirectly, and thus required for biological activity. (See, e.g., Table 1, page 29, and page 66, lines 1-33) The detail with which this information is provided is exemplified by almost ten pages of structure/function relationship description beginning at page 63 of the instant specification. The guidance provided in the specification cannot be trivialized. The atomic coordinates provide the skilled worker with a three dimensional model of the molecule. Bowie, et al., does not take into consideration this degree of knowledge when asserting protein modification unpredictability.

The Examiner's further assertion that "the hydrophobic internal residues are essential for structural integrity" as disclosed at page 66 (Office action at page 5) is essentially correct, but not all helix residues make up the hydrophobic core of the protein. (See, e.g., page 66, lines 9 through 26) Modifications to a helix outside of the hydrophobic core can be made which do not result in inactive variants as exemplified by the modification to lysine at residue 17 which is located in the A helix. Moreover, the specification correlates structural integrity with biological activity (See, e.g., page 66, lines 1-33) and the claims require the recited analog to have biological activity. Thus, modification to any helix residues that result in inactive variants are necessarily outside the scope of the claims.

The Examiner asserts that the claims do not limit the number of residues that can be modified in an analog. Applicant submits that claims of this scope are enabled in view of the guidance imparted in the specification to the worker of ordinary skill, a level of guidance that the disclosure of Bowie, et al., fails to consider. With the disclosure of the atomic coordinates and a computer program, the worker of ordinary skill could make the analogs according to the claims and predict whether any applied change(s) would affect the overall structural integrity/biological activity without undue experimentation. Accordingly, this rejection of claims under §112, first paragraph, may properly be removed.

IV. The rejection of claims 62-66 under 35 USC §102(b), may be withdrawn.

Claims 62-66 were rejected under 35 USC §102(b) as being anticipated by Shaw (US Patent No. 4,904,584). Specifically, the Examiner asserted that Shaw discloses the same mutations recited in claims 62-65. Applicant respectfully traverses.

Claims 62-66 are drawn, in part, to a G-CSF analog comprising "at least one amino acid sequence in an external loop is altered to include one or more lysine amino acid residues, wherein one or more of said lysine amino acid residues is covalently modified with polyethylene glycol (PEG)." The specification teaches that the entire region between helix A and helix B is not a loop region; an important section of this region comprises the AB helix which is important for receptor binding (See, e.g., page 71 of the specification). Furthermore, the specification as filed teaches that the AB loop is located at amino acid residues 58-72 (See, e.g., page 68 of the specification).

Shaw does not describe making lysine modifications in specific tertiary structure components of G-CSF, let alone an external loop. Moreover, Shaw fails to describe a lysine modification within an external loop. The lysine residues disclosed by Shaw, lysines 16, 23, 34, and 40, are not located in the AB loop as defined in the instant specification. Accordingly, the rejection of claims 62-66 under 35 USC §102(b), may be withdrawn.

V. The rejection of claims 62-83 under 35 USC §112, second paragraph, may be withdrawn.

Claims 62-83 were rejected under 35 USC §112, second paragraph, for assertedly being indefinite for failing to particularly point out and claim the subject matter which the Applicant regards as the invention. In response, Applicant points out that claims 62, 66, 75, 76, and 79 have been amended to delete the phrase "containing at least one PEG" and, in claims 62 and 66, replaced it with the phrase "wherein one or more of said lysine amino acid residues is covalently modified with polyethylene glycol (PEG)." Accordingly, the rejection of claims 62-83 under 35 USC §112, second paragraph, may be withdrawn.

CONCLUSION

No fees are believed to be due; however, should any fees be necessary in connection with this document, or should the extension of time fee be inadvertently omitted, the Commissioner is hereby authorized to deduct any such fees from Marshall, Gerstein & Borun, LLP account number 13-2855.

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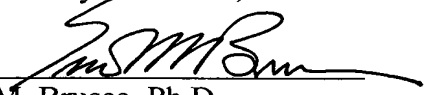
Respectfully submitted,

By 

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